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Root hydrotropism is controlled via a cortex-specific growth mechanism

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Plants can acclimate by using tropisms to link the direction of growth to environmental conditions. Hydrotropism allows roots to forage for water, a process known to depend on abscisic acid (ABA) but whose molecular and cellular basis remains unclear. Here we show that hydrotropism still occurs in roots after laser ablation removed the meristem and root cap. Additionally, targeted expression studies reveal that hydrotropism depends on the ABA signalling kinase SnRK2.2 and the hydrotropism-specific MIZ1, both acting specifically in elongation zone cortical cells. Conversely, hydrotropism, but not gravitropism, is inhibited by preventing differential cell-length increases in the cortex, but not in other cell types. We conclude that root tropic responses to gravity and water are driven by distinct tissue-based mechanisms. In addition, unlike its role in root gravitropism, the elongation zone performs a dual function during a hydrotropic response, both sensing a water potential gradient and subsequently undergoing differential growth.

Tropic responses are differential growth mechanisms that roots use to explore the surrounding soil efficiently. In general, a tropic response can be divided into several steps, comprising perception, signal transduction and differential growth. All of these steps have been well characterized for gravitropism, where gravity-sensing cells in the columella of the root cap generate a lateral auxin gradient, whilst adjacent lateral root cap cells transport auxin to epidermal cells in the elongation zone, thereby triggering the differential growth that drives bending[1–4]. In gravity-stimulated roots, the lateral auxin gradient is transported principally by AUX1 and PIN carriers[3–5].

Compared with gravitropism, the tropic response to asymmetric water availability, hydrotropism, has been far less studied. Previously, it was reported that surgical removal or ablation of the root cap reduces hydrotropic bending in pea[6–8] and *Arabidopsis thaliana*[9], suggesting that the machinery for sensing moisture gradients resides in the root cap. It has also been reported that hydrotropic bending occurs due to differential growth in the elongation zone[7,10]. However, unlike gravitropism, hydrotropism in *A. thaliana* is independent of AUX1- and PIN-mediated auxin transport[11,12]. Indeed, roots bend hydrotropically in the absence of any redistribution of auxin detectable by auxin-responsive reporters[13,14]. Instead, root hydrotropism requires signalling by the hormone abscisic acid (ABA)[12]. These findings imply that, compared to gravitropism, hydrotropism requires a distinct signalling mechanism[15].

The involvement of ABA in hydrotropism was initially suggested by aberrant responses in *A. thaliana* mutants deficient for ABA synthesis or response[12]. More recently, loss-of-function ABA receptor and response mutants that are insensitive or hypersensitive to ABA have been shown to be insensitive or hypersensitive to a hydrotropic stimulus, respectively[16]. In addition, hydrotropism in *A. thaliana* roots requires a gene called *MIZU-KUSSEI1* (*MIZ1*)[17], which is upregulated by application of 10 μM ABA[18]. Despite *miz1* roots being oblivious to water potential gradients, they

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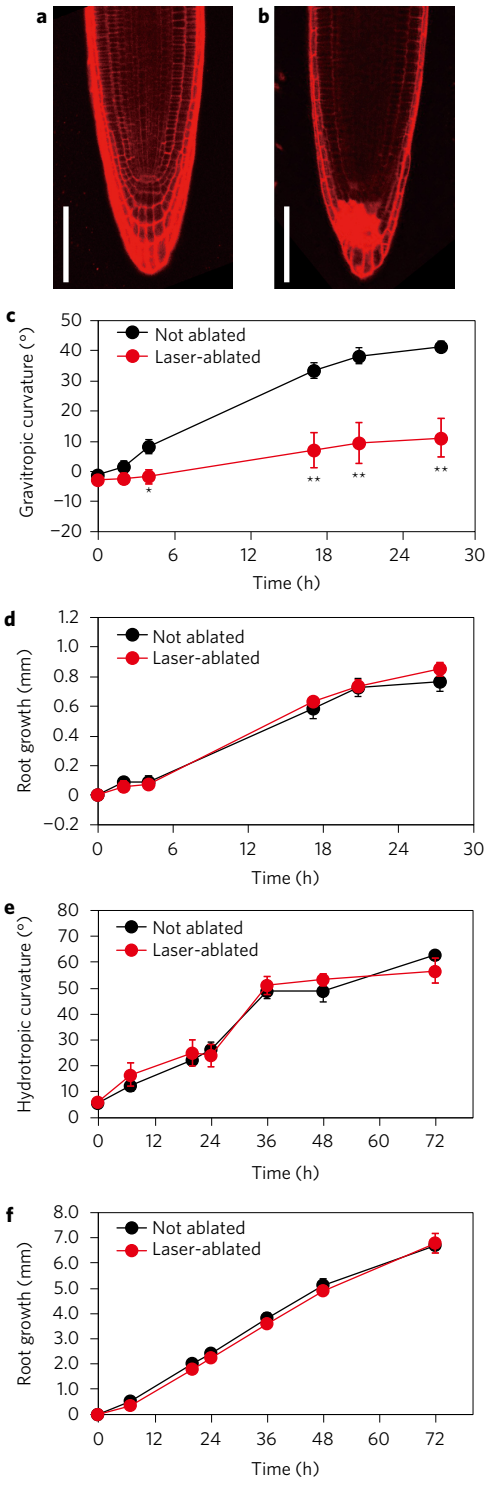

Figure 1 | Laser ablation of columella cells affects the gravitropic but not the hydrotropic response of roots. a,**b**, Confocal fluorescence micrograph of propidium iodide-stained primary root tips before (**a**) and after (**b**) femtosecond-laser ablation of the columella; scale bar, 100 µm. **c**,**d**,Time-course study of root gravitropic curvature (**c**) and root growth (**d**). In **c**, 0° equals horizontal. **e**,**f**, Time-course study of root hydrotropic curvature (**e**) and root growth (**f**). In **e**, 0° equals vertical. The hydrotropism assay was performed using the split-agar system with 812 mM sorbitol. Values are mean ± s.e.m. of a representative experiment, $n = 3$–6, from three independent experiments. Asterisks indicate statistically significant differences (*$P < 0.05$, **$P < 0.01$, Student's *t*-test).

nevertheless bend like wild type in response to gravity[17]. The MIZ1 sequence contains a DUF617 domain that is conserved among the genomes of terrestrial plants, but absent in algae and animals, suggesting a role for hydrotropism in the evolution of land plants[17]. A functional *MIZ1:MIZ1-GFP* fusion protein is expressed in lateral root cap cells as well as cortex and epidermis cells in the meristem and elongation zone[18,19]. However, it is unclear whether this broad expression pattern is necessary for MIZ1's function in hydrotropism or whether ABA signal transduction components in general have to be expressed in specific root tip tissues for a hydrotropic response. The present study describes a series of experiments in *A. thaliana* designed to identify the root tissues essential for a hydrotropic response. We report that MIZ1 and the key ABA signal-transduction component SnRK2.2 expressed specifically in the root cortex are sufficient to drive hydrotropism, and conversely that hydrotropism is blocked by inhibiting the ability of specifically the cortex to execute a differential growth response. Our results support a re-evaluation of hydrotropic signalling, revealing the importance of the cortex and the elongation zone for signal perception as well as bending.

Results

The root meristem and columella are dispensable for hydrotropism. To uncover which root cell types and zones are required during a hydrotropic response in *A. thaliana* roots, we ablated cells using a femtosecond laser. Successful ablation of the columella cells was confirmed by propidium iodide staining of root tissues (Fig. 1a,b) and hydro- and gravitropism assays performed as described previously[11,17] (for details on hydrotropism assays used in this paper, see Supplementary Fig. 1). Whilst columella ablation successfully inhibited the gravitropic response as previously reported[1], it did not inhibit the hydrotropic response (Fig. 1c,e). We suggest that the discrepancy with earlier experiments arose from their being performed under adverse growth conditions, as indicated by roots elongating more than an order of magnitude slower than those used here. Importantly, the ablated roots in this study elongated at an equivalent rate as the intact roots throughout both gravitropism and hydrotropism assays (Fig. 1d,f). Further probing of the region necessary for stimulus perception showed that even when ablation encompassed essentially the entire meristem, hydrotropism was scarcely affected (Supplementary Fig. 2). Crucially, when seedlings with ablated root cap or meristem were placed in an assay system that lacked the moisture gradient, ablated roots responded in the same way as intact roots with only minimal bending, demonstrating that laser ablation *per se* did not induce a response that mimicked hydrotropism (Supplementary Fig. 2).

Because this apparent dispensability of the columella conflicts with previous results with laser ablation[9], we independently validated the experiment in the split-agar system by excising the distal region of the root tip (~250 µm) manually. As with laser ablation, manual excision of columella and meristem did not induce bending in the absence of a water potential gradient (Supplementary Fig. 2), demonstrating that root tip removal does not mimic a hydrotropism response. Most significantly, manual excision of columella and meristem did not disrupt hydrotropic bending in the presence of a water potential gradient, giving results comparable to whole roots (Supplementary Fig. 2). Whilst we cannot exclude the possibility that hydrotropic stimuli are perceived in the root cap when that tissue is present, our ablation and excision results demonstrate that roots are able to sense as well as respond to water potential gradients within the elongation zone.

Root hydrotropism depends on the ABA signalling component SnRK2.2. ABA represents a critical signal for numerous plant

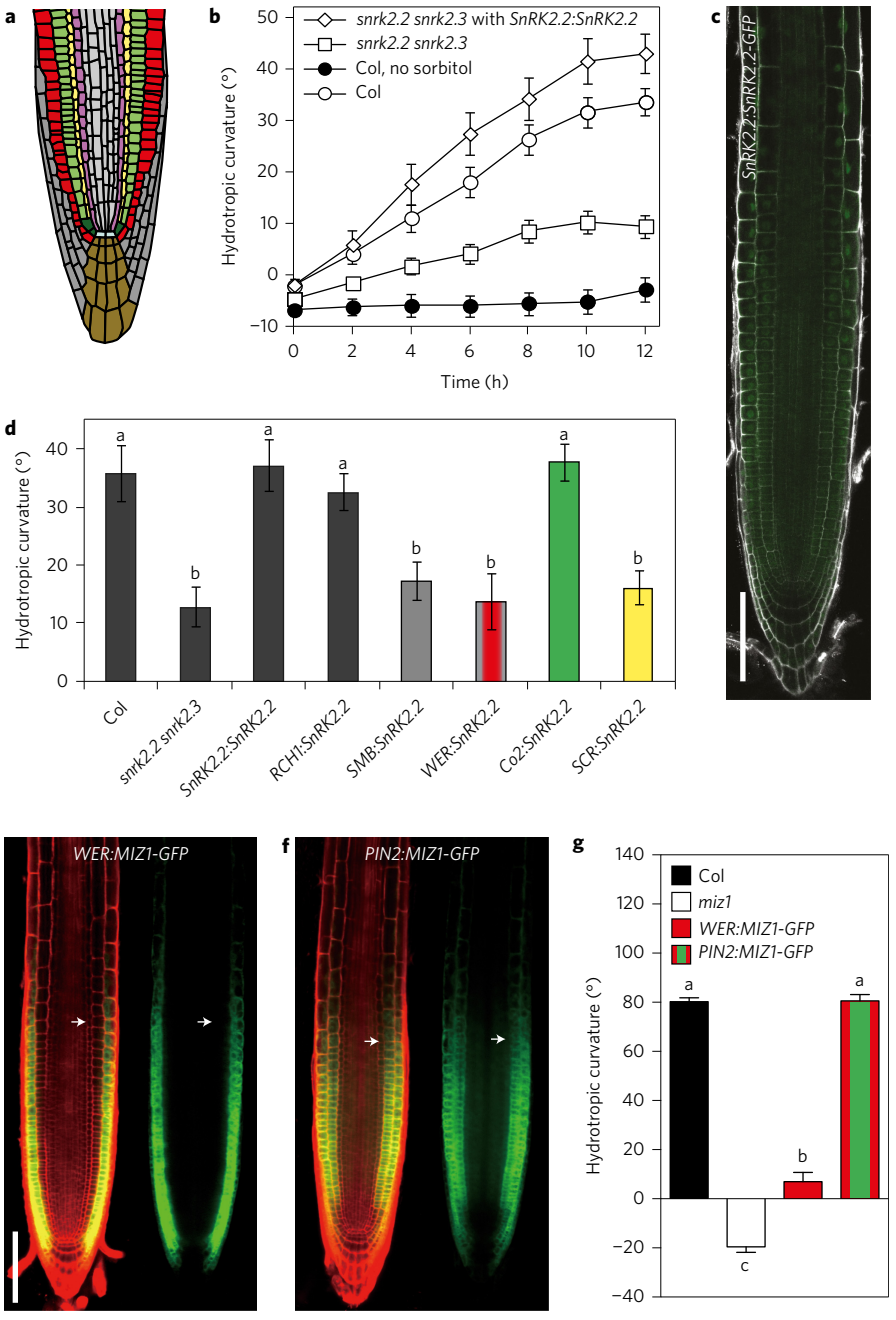

Figure 2 | ABA signalling in the cortex is crucial for root hydrotropism. a, Schematic drawing indicating tissues in the root tip; grey: lateral root cap, red: epidermis, green: cortex, yellow: endodermis. **b**, Kinetics of hydrotropic curvature after transferring seedlings to split-agar plates with 400 mM sorbitol. Values are mean ± s.e.m., $n = 29$–40. **c**, Expression of *SnRK2.2:SnRK2.2-GFP* in the root tip; scale bar, 100 µm. **d**, Hydrotropic curvature 12 h after transfer to split-agar plates with 400 mM sorbitol. Values are mean ± s.e.m., $n = 24$–31. Different letters indicate statistically significant differences ($p < 0.05$, Fisher's least significant difference (LSD)). **e**,**f**, Expression pattern of MIZ1-GFP fusion protein under control of (**e**) the *WER* and (**f**) *PIN2* promoters with *HSP* terminator. Left-hand image shows an overlay of fluorescence from GFP (green) and propidium iodide (red), right-hand image shows GFP only. Arrow indicates the approximate rootward boundary of the elongation zone; scale bar, 100 µm. **g**, Hydrotropic curvature 12 h after transfer of seedlings to the moisture gradient in air assay system. Values are mean ± s.e.m. of three independent experiments, $n = 35$–44. Different letters indicate statistically significant differences ($P < 0.05$, Tukey honest significant difference (HSD) test). Col, *A. thaliana* Columbia-0 accession.

abiotic stress responses[20] including root hydrotropism. ABA responses are mediated by a negative regulatory signalling module involving soluble receptors of the START-domain superfamily (PYR/PYL/RCARs), clade A, type 2C protein phosphatases (PP2Cs) and subclass III Snf1-related kinases (SnRK2s)[20]. ABA binds to PYR1/PYL/RCAR, which induces a conformational change that allows the receptor proteins to bind to, and thereby inhibit, PP2Cs[21,22]. PP2Cs dephosphorylate SnRK2s, suppressing their activity; thus, SnRK2 activity increases in the presence of ABA due to PP2Cs being bound to the PYR1/PYL/RCAR ABA receptors[23]. When active, the SnRK2s phosphorylate transcription factors and other downstream targets[20,23].

To investigate how ABA controls hydrotropism, we characterized a double mutant lacking the ABA signalling kinases SnRK2.2 and SnRK2.3 (ref. 24). Although retaining some ABA responsiveness, this double mutant was selected for experiments because, in contrast

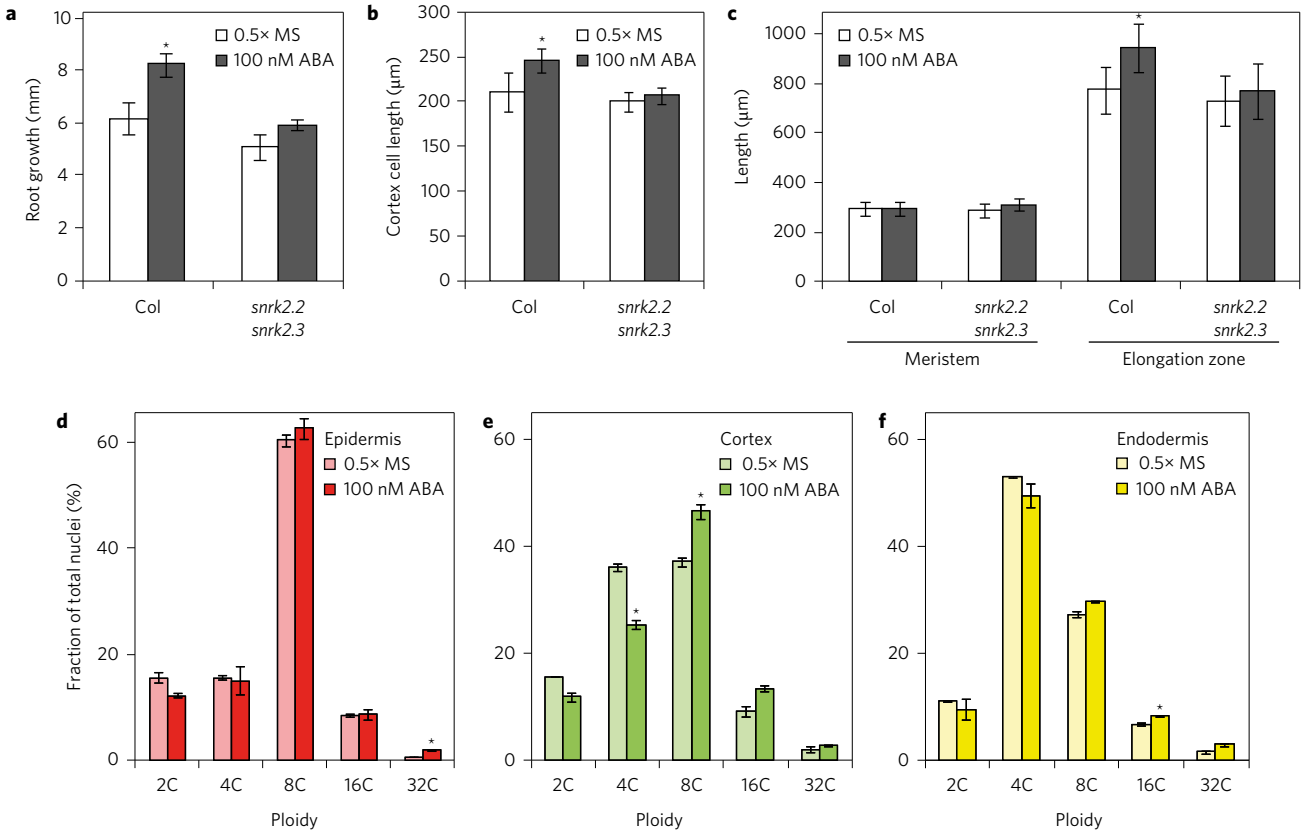

Figure 3 | Root growth and cortical endoreplication are induced by low levels of ABA. a–c, Root growth and histology. **a**, Root growth without (0.5× MS) or with 100 nM ABA 24 h after transfer. Values are mean of three experiments ± s.d., $n = 12–40$. **b**,**c**, Seedlings treated as in **a** were stained with propidium iodide and images taken with a confocal microscope. **b**, Cell length of mature cortex cells. Values are mean ± s.d., $n = 18–47$ cells for ten roots per line and treatment. **c**, Meristem length was determined using Cell-o-Tape and an arithmetic method to determine the meristem end. Length of the elongation zone was determined by measuring the distance from the end of the lateral root cap until the first root hair bulge. Values are mean ± s.d., $n = 11–28$. For **a–c**: *statistically significant difference ($P < 0.01$, Student's *t*-test). **d–f**, Endoreplication. DNA content of nuclei isolated from (**d**) the epidermis (non-hair cells), (**e**) cortex and (**f**) endodermis of roots treated for 24 h without (0.5× MS, light bars) or with 100 nM ABA (dark bars). Values are mean ± s.d. For **d–f**: *statistically significant difference ($P < 0.05$, Student's *t*-test). Col, *A. thaliana* Columbia-0 accession..

to most mutants in ABA perception, it is neither dwarfed nor wilty. We initially assayed hydrotropism in a split-agar-based system[25]. Hydrotropism in the *snrk2.2 snrk2.3* double mutant was strongly attenuated, but was restored in the *snrk2.2 snrk2.3* double mutant expressing the *SnRK2.2* gene under the control of its own promoter (Fig. 2b). Identical results were obtained using a moisture gradient in air hydrotropic assay (Supplementary Fig. 5). Hence, the SnRK2.2 kinase appears to be required for hydrotropism.

As the *snrk2.2 snrk2.3* double mutant had slightly shorter roots and a reduced growth rate compared to wild type (Supplementary Fig. 3), we compared the growth rates of the double mutant on half-strength Murashige and Skoog (MS) medium and hydrotropism plates and found them to be comparable (Supplementary Fig. 3), ruling out hypersensitivity of the *snrk2.2 snrk2.3* double mutant to sorbitol. In addition, we performed split-agar hydrotropism assays with younger wild-type seedlings to assess whether a reduction in tip angle was caused simply by a reduced root growth rate. Roots bent with similar kinetics despite differences in length and growth rate, indicating that hydrotropic bending is not proportional to root growth rate (Supplementary Fig. 3).

Hydrotropism requires SnRK2.2 signalling only in the root cortex. To gain insight into the tissue specificity of hydrotropism, we created a translational GFP fusion to the *SnRK2.2* genomic sequence and expressed the reporter in the *snrk2.2 snrk2.3* double mutant background. In the resulting lines, roots regained wild-type sensitivity to 10 µM ABA (Supplementary Fig. 4) and bent hydrotropically in the moisture gradient in air assay (but not the split-agar assay) (Supplementary Fig. 4). We assume that the differences in hydrotropic response obtained using the different assays could be due to the moisture in air gradient providing a steeper water potential gradient than the split-agar assays. Hence, the translational reporter appeared partially functional. Using confocal imaging, SnRK2.2-GFP signal was detected in nuclear and cytoplasmic compartments, consistent with the sub-cellular localisation of its known regulatory targets[26,27]. Moreover, at the tissue scale, *SnRK2.2:SnRK2.2-GFP* was ubiquitously expressed throughout the root apex, including root cap and elongation zone (Fig. 2c).

To pinpoint the root tissue where SnRK2.2 is required during a hydrotropic response, we expressed the *SnRK2.2* genomic sequence in the *snrk2.2 snrk2.3* double mutant background using a suite of tissue- and zone-specific promoters. *SnRK2.2* expressed under the control of the meristem and transition zone-specific *RCH1* promoter[28] complemented the *snrk2.2 snrk2.3* hydrotropic defect (Fig. 2d). Surprisingly, rescue failed when *SnRK2.2* was expressed specifically in the root cap (SOMBRERO[29], *SMB:SnRK2.2*), epidermis and lateral root cap (WEREWOLF[30], *WER:SnRK2.2*) or endodermis (SCARECROW[31], *SCR:SnRK2.2*) (Fig. 2d). By contrast, double mutant roots bent hydrotropically as the wild type when expressing *SnRK2.2* in just the cortex (Co2 (ref. 32), *Co2:SnRK2.2*) (Fig. 2d). *SnRK2.2* expression levels in the *Co2:SnRK2.2* line were

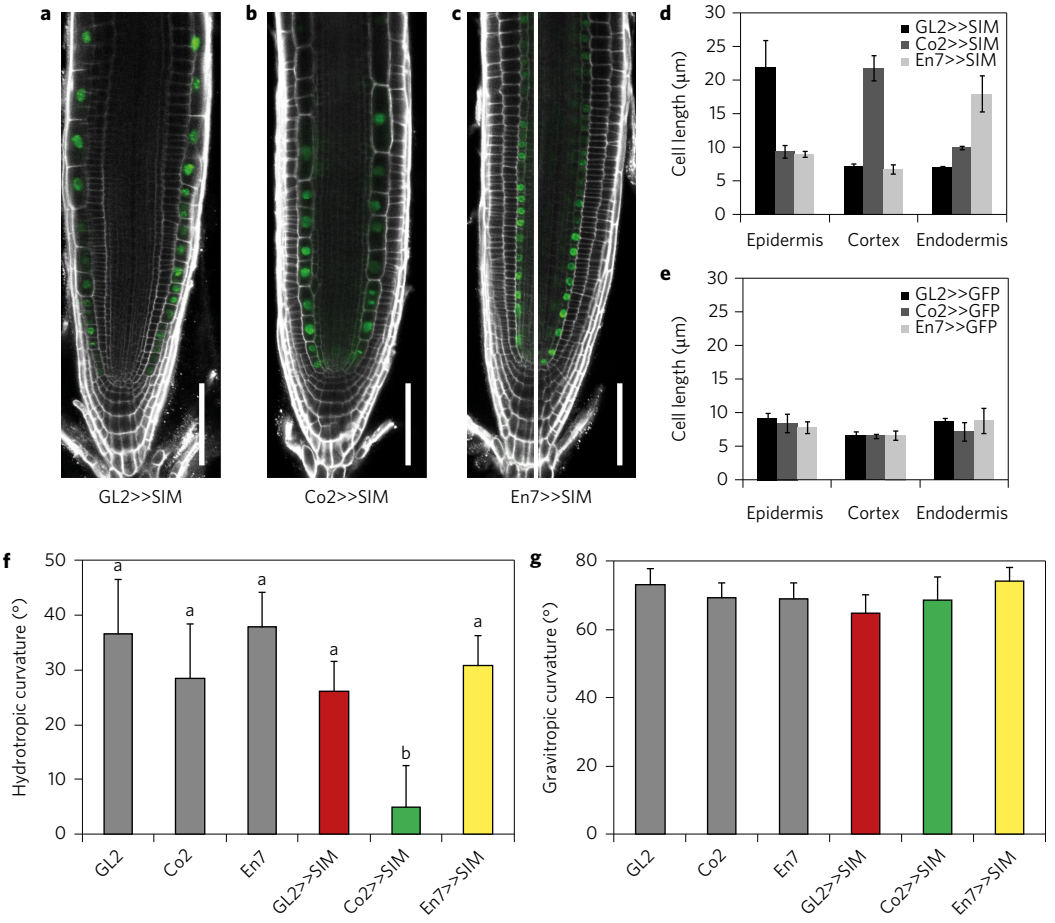

Figure 4 | Inhibition of differential cell elongation in the cortex prevents hydrotropism but not gravitropism. a–c, Confocal images of root tips co-expressing *SIM* and *NLS-GFP* (green) in (**a**) epidermis, (**b**) cortex and (**c**) endodermis. Cell walls were stained with propidium iodide (white). In **c**, two images of the same root are shown for better visualization of the endodermis cell file. Scale bars for **a–c**, 100 µm. **d**,**e**, Quantification of cell lengths for epidermis, cortex and endodermis files in the meristem. Values are mean ± s.d., $n = 7–52$ cells from three plants for each line and tissue. **f**, Hydrotropic curvature 10 h after transfer to split-agar plates with 400 mM sorbitol. Values are mean ± 2 × s.e.m., $n = 14–15$ for parental lines (GL2, Co2, En7) and $n = 56$ for *SIM* expression lines (GL2>>SIM, Co2>>SIM, En7>>SIM). Different letters indicate statistically significant differences ($P < 0.05$, Fisher's LSD). **g**, Gravitropic curvature 8 h after plates were rotated by 90°. Values are mean ± 2 × s.e.m., $n = 30–31$.

low in comparison to non-rescuing epidermal, lateral root cap or endodermal driven lines, demonstrating that mutant rescue is not simply a dose effect (Supplementary Fig. 5). In addition, we confirmed the hydrotropism response of the *Co2:SnRK2.2* line using the moisture in air gradient assay (Supplementary Fig. 5). Hence, root hydrotropism appears to require the ABA response machinery specifically in the cortex.

Cortex-specific MIZ1 expression rescues the *miz1* hydrotropic defect. To independently assess tissue specificity for the hydrotropic response, we determined which tissues require MIZ1, a protein previously identified as essential for hydrotropism and localized to cortex, epidermis and lateral root cap[19]. We used various promoters to express MIZ1-GFP in specific tissues in the *miz1* background (Supplementary Fig. 6). When constructs that included the *MIZ1* terminator were used, MIZ1-GFP expression driven by *RCH1* was detected in the meristem, by *SMB* in the root cap, by *SCR* in the endodermis and by *COR* and by *Co2* in the cortex, all as expected[28,29,31–33] (Supplementary Fig. 6). Compared to *SCR* or *Co2*, the *COR* promoter drove MIZ1-GFP expression farther into the elongation zone. By contrast, the *WER* promoter drove MIZ1-GFP expression not only in the epidermis and lateral root cap, as expected[30], but also in the cortex. Like *COR*, expression from *WER* continued well into the elongation zone. Note that none of these constructs altered root growth rate appreciably (Supplementary Fig. 6).

Using the tissue-specific MIZ1-GFP constructs, we assayed hydrotropism using the moisture gradient in air method, which gave approximately 80° bending after 12 h. As expected, hydrotropic bending was fully rescued by expressing MIZ1-GFP under the *MIZ1* promoter (Supplementary Fig. 6). By contrast, little or no hydrotropic curvature resulted when MIZ1-GFP was expressed in root cap (*SMB*), in endodermis (*SCR*) or in the meristem (*RCH1*). Mutant complementation was only partial using *Co2* to drive MIZ1-GFP expression, but rescue was complete employing either *WER* or *COR* promoters, revealing a requirement for MIZ1 in the elongation zone (Supplementary Fig. 6). Mutant rescue was also complete when MIZ1-GFP expression was driven by the *PIN2* promoter, which, like *WER*, drives expression in lateral root cap, epidermis and cortex, which for the latter tissues continues well into the elongation zone (Fig. 2e–g). Finally, when *WER*-driven expression was removed from the cortex, which happened if the native MIZ1 terminator was replaced by a terminator from a heat-shock protein (HSP), *miz1* rescue essentially failed (Fig. 2e,g). Identical responses for *WER*- and *PIN2*-driven MIZ1-GFP expression were obtained using the split-agar assay (Supplementary Fig. 5). Taken together, these results show that hydrotropic bending requires MIZ1 expression specifically in the root cortex and that the

expression domain must span at least part of the elongation zone. This conclusion is consistent with laser ablation and SnRK2.2 expression experiments that, when taken collectively, establish the functional importance of the cortex within the elongation zone for the hydrotropic response.

Low levels of ABA promote root elongation. Root cortical cells abut the endodermis (Fig. 2a), a recently reported site of ABA accumulation in roots[34]. Hence, ABA response machinery in the cortex would be ideally positioned to sense lateral movement of ABA from the endodermis into outer root tissues, presumably triggering growth responses. In roots, whilst high ABA levels inhibit growth[24], low levels of this hormone promote elongation at low water potential[35–37]. To understand the ABA-dependent growth mechanism underlying hydrotropism, we next investigated the effect of low doses of ABA on root growth. Transferring seedlings onto 100 nM ABA stimulated root growth rate in the wild type but had minimal effect on *snrk2.2 snrk2.3* (Fig. 3a and Supplementary Fig. 7). Comparing meristem and elongation zone of those roots, 100 nM ABA appeared to change neither the length nor cell number within the meristem but significantly increased the length of the elongation zone in wild type and *Co2:SnRK2.2* complementation lines (Fig. 3c and Supplementary Fig. 7). The increased root growth rate was accompanied by both an increased rate of cell production and an increased mature cell length (Supplementary Fig. 7 and Fig. 3b). Taken together, these data suggest that low doses of ABA in these non-stressed plants stimulate rates of cell division and elemental elongation.

To examine tissue specificity in the promotion of root growth by ABA, we analysed nuclear ploidy of specific tissues by performing cell sorting and DNA-content measurements. Significantly, 100 nM ABA stimulated endoreplication specifically in root cortical cells, as evidenced by the increased fraction of 8C nuclei at the expense of 4C (Fig. 3e). By contrast, 100 nM ABA had little if any effect on endoreplication in either atrichoblast or endodermal cells (Fig. 3d,f). Hence, ABA appears to specifically trigger changes in cell cycle machinery in just the cortex, consistent with a fundamental role for this tissue in mediating hydrotropism.

Hydrotropism is driven by differential cortical cell expansion. One might question whether an asymmetry of growth-promoting mechanisms within a single tissue could provide sufficient mechanical leverage to trigger root curvature. To explore whether such changes in the dynamics of cortical cells are sufficient to drive root bending during hydrotropism, we developed a mathematical model (see Methods and Supplementary Note 1), taking advantage of recent theoretical work that successfully recapitulates the root's growth rate profile by ascribing distinct mechanical contributions to the various tissues[38]. For a short period following exposure to the water potential gradient, a small group of cortical cells on the dry side of the root were treated as undergoing early entry into rapid elongation, changing their mechanical properties to be the same as cells in the elongation zone. This differential elongation, coupled with the cell-to-cell adhesion typical for plant cells, caused the root midline to bend in this region (Supplementary Fig. 8).

To assess the model further, we quantified the growth kinetics of hydrotropically bending roots by using image analysis, resolving both elemental elongation and curvature. These experimental data resembled the evolution of root tip angle predicted by the model (Supplementary Fig. 8). Hence, the root cortex emerges as a plausible driver to accomplish hydrotropic bending. Taken together, the experimental data and model simulations support our hypothesis that hydrotropism is driven by differential elemental expansion within the root cortex.

If hydrotropic bending is driven by an asymmetric expansion of cortical cells in the elongation zone, we reasoned that hydrotropism could be blocked by interfering with the orderly progression of cells through the growth zone. To test this, we took advantage of the overexpression phenotype of the cyclin-dependent kinase inhibitor SIAMESE (SIM), in which cell division is inhibited and endoreplication is stimulated[39]. We used a GAL4-VP16-driven transactivation system to co-express SIM and a nuclear-localized GFP marker specifically in either epidermis, cortex or endodermis. In each case, root meristem cells overexpressing SIM were enlarged (Fig. 4a–c) but cells in adjacent tissues were not detectably affected and were of similar length to cells of roots expressing only the *GFP* marker (Fig. 4d,e). Next, we tested each tissue-specific, *SIM*-overexpressing line for hydrotropism. Roots overexpressing *SIM* in root epidermis or endodermis bent indistinguishably from the parental lines, whereas *SIM* overexpression in the cortex blocked root hydrotropic bending (Fig. 4f). By contrast, roots of every *SIM* overexpression line retained a wild-type response to gravity (Fig. 4g), revealing that *SIM* overexpression in the cortex did not simply prevent all differential root growth processes.

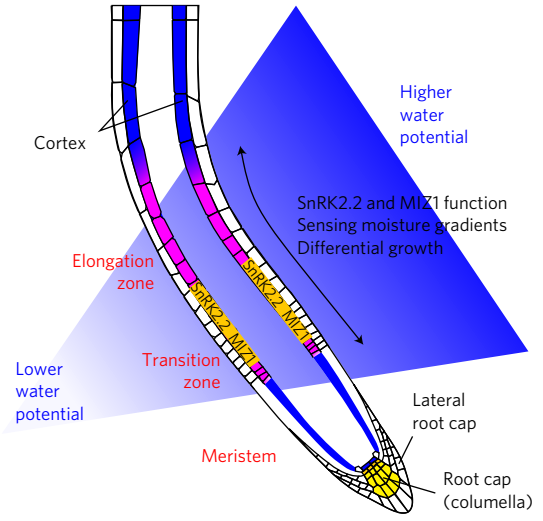

Figure 5 | Conceptual model for root hydrotropism. SnRK2.2 and MIZ1 expression in cortex cells of the transition and elongation zone are required to mediate the ABA-dependent differential growth response to a water potential gradient. Perception of the water potential gradient does not require tissues in the root cap or meristem, but takes place in the transition and elongation zones where the differential growth response occurs.

Discussion

We report that root tropic responses to gravity and water are driven by distinct molecular and tissue-based mechanisms. In the case of gravity, root re-orientation is sensed by columella cells at the root tip[1], triggering the formation of a lateral auxin gradient across the root with higher concentrations on the lower side of the root[40,41]. This auxin gradient is then transported via the lateral root cap to epidermal cells in the elongation zone[3] where it elicits downward root bending by stimulating expansion on the upper side and inhibiting it on the lower-side[42]. By contrast, here, laser ablation experiments demonstrate that perceiving a water potential gradient and fully responding thereto requires neither meristem, nor lateral root cap nor columella (Fig. 1). Hence, unlike its role in root gravitropism, the elongation zone is able to perform a dual function during a hydrotropic response, both sensing a water potential gradient and undergoing differential growth. This conclusion stands despite the possibility of meristem and root cap participating in hydrotropism in intact roots, for example by integrating signals from water and gravity.

We also confirm that root hydrotropism uses the hormone ABA and that the ABA signal transduction components SnRK2.2 and

SnRK2.3 play a key role in regulating root re-orientation. Surprisingly, targeted *SnRK2.2* expression studies in *snrk2.2 snrk2.3* (Fig. 2) revealed the critical importance during hydrotropism of ABA response machinery just in the cortex. The importance of this specific root tissue for hydrotropism was further supported by the response depending on cortical expression of MIZ1 (Fig. 2). Taken together, our results demonstrate that ABA and MIZ1 responses in the cortex of the root elongation zone play a central role in the hydrotropic response of *A. thaliana* roots (Fig. 5). Hence, root gravitropic and hydrotropic responses are driven by distinct signals and tissue-based mechanisms. Consistent with our conclusion, Krieger *et al.*[43] recently described the opposing effect of reactive oxygen species on these tropic responses and the distinct positions at which roots bend during gravitropic and hydrotropic responses.

A key question for hydrotropic research is to understand how a modest gradient in water potential across the root is perceived (and presumably amplified) into a growth response. Mechanosensing, differential movement of water, ions or signalling molecules all represent likely candidates, but detection methods that are more sensitive than those currently available will be necessary to get to the root of this plant environmental response.

Methods

Ablation of root-tip cells using laser-microscopy systems. For micro-beam laser irradiation, 4-day-old seedlings were aligned in a micro-chamber comprising two glass coverslips (25×60 mm^2 and 24×24 mm^2, Matsunami) and a seal (TaKaRa Slide Seal for *in situ* PCR, Takara Bio). The micro-chamber was filled with low-melting agar (0.5× MS medium, 0.4% (w/v) sucrose (Wako Pure Chemical Industries), 0.2% (w/v) low-melting agarose (SeaPlaque; FMC BioProducts)). These samples were put on the stage of a microscope (Nikon ECLIPSE TiE, Nikon) and irradiated with an N_2 pulsed micro-beam laser through Coumarin 440 with an averaged power of 330 kW for a 3–5 nanosecond pulse (MicroPoint PIJ-3-1; Andor Technology). For femtosecond-laser irradiation, seedlings were placed on 0.5× MS medium on a glass slide. Amplified femtosecond laser pulses from a regeneratively amplified Ti:sapphire femtosecond laser system (IFRIT; 780 ± 5 nm, 230 fs, <1 mJ per pulse, 1 kHz, Cyber Laser Inc.) were focused onto root cap cells through a ×10 objective lens (UPlanSApo NA 0.4, Olympus) on a confocal laser scanning microscope (FV1000-BX51, Olympus). Laser pulses (200) were detected with a mechanical shutter (gate time: 200 ms) and delivered to the sample. The laser pulse was collimated by dual convex lenses before the microscope and the laser focal point was tuned to the plane of the image. The diameter of the laser focal point, which is consistent with the beam waist, was about 1 μm. A neutral density filter was put between the laser and microscope and used to tune the laser pulse energy to around 400 nJ per pulse, which is about four times larger than the threshold energy for cavitation bubble generation in water (100 nJ per pulse). Laser-ablated seedlings were incubated on 0.5× MS medium for 1 h in a vertical position before performing further assays.

Root tropism and growth assays. The hydrotropism assays shown in Fig. 1 and Supplementary Fig. 5b–e were performed as described previously using a split-agar system with 812 mM sorbitol[11]. Gravitropism assays shown in Fig. 1 and Supplementary Fig. 2 were performed using 1% agar medium with or without 0.5× MS medium as described previously[17]. Hydrotropism assays shown in Fig. 2g and Supplementary Figs 2g–l, 4e,f, 5f,g and 6 were performed using a moisture gradient in air as described previously[11]. Four-day-old seedlings were used for all tropism assays described above.

Hydrotropism assays shown in Figs 2b,d and 4 and Supplementary Figs 2m, 3, 4d and 8 were performed as previously described[25] using 5-day-old seedlings in a split-agar system with 400 mM sorbitol.

For gravitropism assays shown in Fig. 4, 5-day-old seedlings were transferred to new plates containing 0.5× MS medium with 1% agar. After acclimatisation for 2 h in the controlled environment room, plates were rotated by 90°. Images of seedlings were acquired using an automated imaging platform[44] and root tip angle and length determined using the Fiji image processing package (http://fiji.sc/Fiji).

For assessing root growth response to ABA, 5-day-old seedlings were transferred to new plates containing 0.5× MS medium with the indicated amount of ABA (Sigma). To determine meristem cell number and length, longitudinal images of root tips clearly showing the cortex cell file were taken with a confocal laser scanning microscope, using propidium iodide to stain cell walls. Starting from the quiescent centre, the length of individual cortex cells was determined using the Cell-o-Tape macro[45] for Fiji. The mean length of meristem cells was calculated using ten cells from the rapid amplifying region of the meristem (cells 10–19 counting shootward from the quiescent centre) and the end of the meristem deemed to have been reached when consecutive cells had reached or exceeded the mean length by two. Cell production rates were calculated as previously described[46].

Modelling root bending. A mechanical model has been developed to describe hydrotropism-associated root bending. The approach[38] exploits the large aspect ratio of the root, which allows a relatively simple description of bending in terms of the stretch and curvature of the root midline. A viscoplastic constitutive relation is adopted (viscous flow where the yield stress is exceeded), with the yield stress of cortical cells on the dry side of the root modified in response to a hydrotropic stimulus; the resulting partial differential equations for the dependence of midline stretch and curvature in terms of time and arc length are solved numerically by a finite-difference approach. Further details are given in the Supplementary Note 1, Section 2.

Data availability. The data that support the findings of this study are available from the corresponding authors upon request.

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Author contributions

D.D., L.P., A.K., J.F., V.B., R.B., R.A., T.N., S.H., T.-W.B., Y.M., D.M.W., S.T. and C.J.S. performed experimental work and data analysis and mathematical modelling. D.M.W., M.R.O., L.R.B., R.D., O.J., J.R.K., S.J.M., J.R., R.B., J.D., P.L.R., T.I.B., T.P., L.D.V., N.F., Y.M., A.N., Y.H., H.T. and M.J.B. oversaw project planning and discussed experimental results and modelling simulations. D.D., L.P., A.K., N.F., Y.M., T.I.B., H.T. and M.J.B. wrote the paper.

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Competing interests

The authors declare no competing financial interests.